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Structure–activity relationships of carbocyclic 6-benzylthioinosine analogues as subversive substrates of *Toxoplasma gondii* adenosine kinase

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ABSTRACT

Carbocyclic 6-benzylthioinosine analogues were synthesized and evaluated for their binding affinity against *Toxoplasma gondii* adenosine kinase [EC.2.7.1.20]. Various substituents on the aromatic ring of the 6-benzylthio group resulted in increased binding affinity to the enzyme as compared to the unsubstituted compound. Carbocyclic 6-(*p*-methylbenzylthio)inosine **9n** exhibited the most potent binding affinity. Docking simulations were performed to position compound **9n** into the *T. gondii* adenosine kinase active site to determine the probable binding mode. Experimental investigations and theoretical calculations further support that an oxygen atom of the sugar is not critical for the ligand-binding. In agreement with its binding affinity, carbocyclic 6-(*p*-methylbenzylthio)inosine **9n** demonstrated significant anti-toxoplasma activity (IC_{50} = 11.9 μM) in cell culture without any apparent host-toxicity.

1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects a broad range of warm-blooded animals including humans.[1–3] The prevalence of toxoplasmic infection is estimated to be a billion people throughout the world.[4,5] Toxoplasmic infection is generally asymptomatic and self limiting because it is controlled by the immune system of healthy individuals. However, *T. gondii* causes severe disease in immunocompromised patients with AIDS, organ transplant and leukemia.[6,7] During pregnancy, infection with *T. gondii* can be transmitted to the fetus leading to congenital toxoplasmosis of the fetus.[8,9]

Current therapeutic regimens are antifolate drugs such as the combination of pyrimethamine and sulfadiazine, which synergistically inhibits folate metabolism.[7,10,11] Unfortunately, this therapy is not effective against *T. gondii* tissue cysts and should not be used in pregnant women. Furthermore, this treatment causes side effects such as bone marrow depression and skin rashes attributed to the sulfa drugs,[7,9,10] which frequently result in discontinuation of this therapy. Therefore, their application has been hampered by limited effectiveness, host-toxicity and drug-resistance. Hence, there is a continuing need to develop new and effective chemotherapeutic agents for long-term treatment of toxoplasmosis.

T. gondii adenosine kinase (EC.2.7.1.20) is a major enzyme of purine metabolism in the parasite. Differences between the characteristics of *T. gondii* adenosine kinase and the mammalian enzyme render *T. gondii* adenosine kinase an excellent chemotherapeutic target.[12,13] We have recently reported structure–activity relationship studies of 6-benzylthioinosine,[14–16] N^6-benzyladenosine[17] and 7-deaza-6-benzylthioinosine analogues[18,19] as substrates of *T. gondii* adenosine kinase. We have also reported that 6-benzylthioinosine[15,16,20,21] and 7-deaza-6-benzylthioinosine analogues[18,19] are selectively toxic to *T. gondii* infected cells but not uninfected host cells. In spite of the similarity in their structures, and unlike the 6-benzylthioinosines, most of the N^6-benzyladenosine analogues were toxic to both infected and uninfected cells.[17] These studies demonstrated that the sulfur atom on the 6-position of the inosine motif is indispensable for the selective binding to *T. gondii* adenosine kinase, which results in selective toxicity against the parasite. It is noteworthy that all of these aforementioned compounds are ribonucleosides, consisting of a ribose ring as the sugar moiety.

As part of our continuing efforts to develop potent and selective anti-toxoplasmic agents, we turned our attention to carbocyclic nucleosides, wherein an oxygen atom of the ribose ring in 6-benzylthioinosine analogues (**II**) is replaced by a methylene group to make the corresponding carbocyclic nucleosides (**I**) (Fig. 1). One of the advantages of the carbocyclic nucleosides is their metabolic stability due to the absence of a typical glycosidic bond.[22,23] The carbocyclic nucleosides also have considerable effects on ring conformation and lipophilicity which may improve their therapeutic potency.[22,23] Specifically, we wanted to investigate the structural effects of the carbasugar moiety on the binding of 6-benzylthioinosine to *T. gondii* adenosine kinase. Other molecular alterations were

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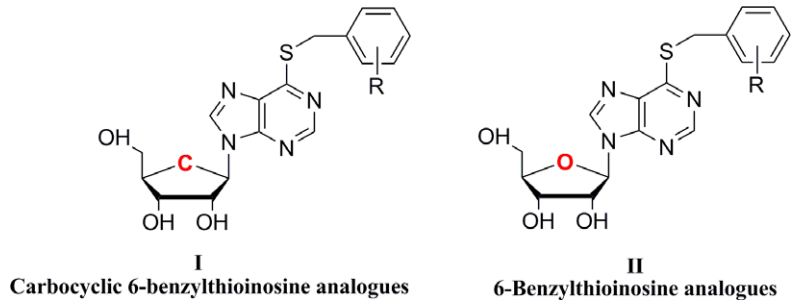


Figure 1. Chemical structures of carbocyclic 6-benzylthioinosine and 6-benzylthioinosine analogues.

also accompanied by introducing substituents on the aromatic ring of the 6-benzylthio group of this carbocyclic nucleoside. It was of interest to ascertain how the carbasugar moiety would be accommodated within the *T. gondii* adenosine kinase active site in conjunction with the glide XP docking studies. Herein, we report the synthesis, structure–activity relationships and molecular modeling studies of novel carbocyclic 6-benzylthioinosines as well as the structural effects of the carbasugar moiety on their anti-toxoplasmic activities.

2. Results and discussion

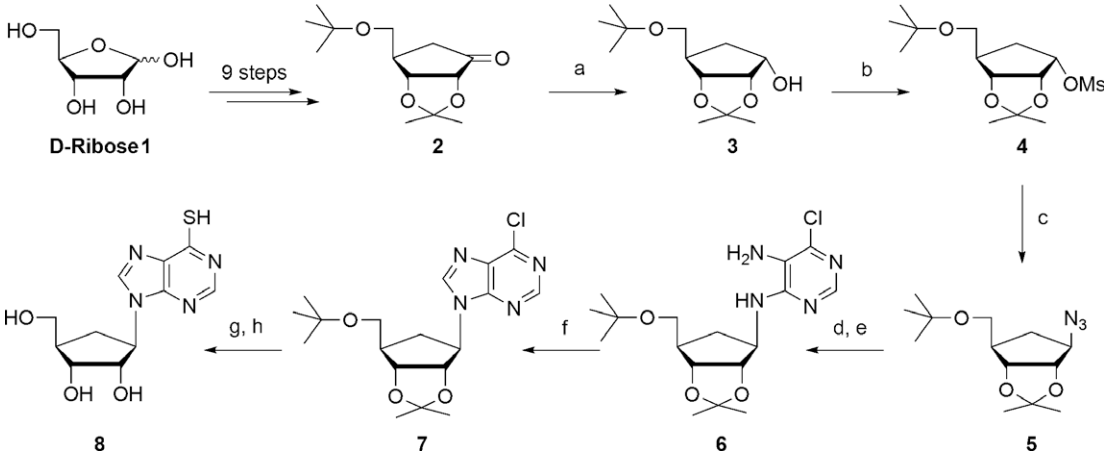
2.1. Chemistry

We synthesized a series of carbocyclic 6-benzylthioinosine analogues for structure–activity relationships on *T. gondii* adenosine kinase as shown in Schemes 1 and 2. Compound **2** was prepared from D-ribose **1** in nine steps using previously developed methodology in our laboratory, which allowed the preparation of multigram quantities of cyclopentanone **2**.[24–26] Compound **2** was stereoselectively reduced with sodium borohydride to provide the corresponding alcohol **3** in quantitative yield. Compound **3** was treated with methanesulfonyl chloride in the presence of Et_3N to afford compound **4**, which was then displaced with sodium azide in DMF at 150 °C to give carbocyclic azide **5**. Reduction of compound **5** with 10% palladium on carbon at 30 psi under hydrogen atmosphere,[27] followed by coupling with 5-amino-4,6-dichloropyrimidine produced compound **6**. The 6-chloropurine derivative **7** was prepared by cyclization of the compound **6** with ethyl orthoformate and a catalytic amount of *p*-toluenesulfonic acid.[24] Treatment of compound **7** with thiourea in ethanol provided the corresponding thione,[28] which was deprotected with aqueous trifluoroacetic acid to give a 6-mercaptopurine derivative **8**. Carbocyclic nucleoside analogues **9a**–**u** were synthesized via solution phase parallel synthesis (Scheme 2).[29,30] The thione **8** served as a key intermediate, which was alkylated with appropriate benzyl halide to afford target compounds **9a**–**u** in good yields after short column purification.

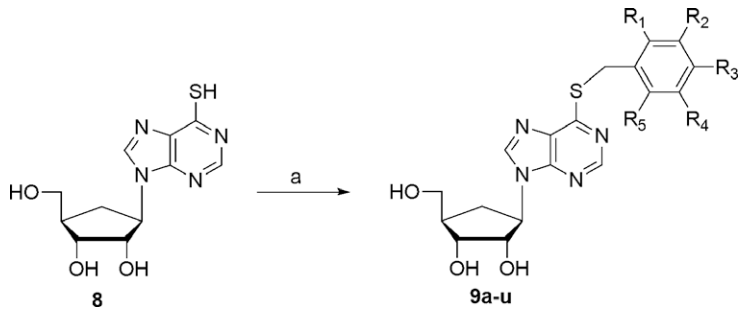
2.2. Structure–activity relationships

Carbocyclic 6-benzylthioinosine (**9a**) and its analogues (**9b**–**u**) were assayed for their binding affinity to purified *T. gondii* adenosine kinase, and the results are shown in Table 1. In the previous studies,[18] replacement of the nitrogen atom at the 7-position by CH function in the purine ring enhanced the binding affinity of 6-benzylthioinosine to *T. gondii* adenosine kinase, providing the flexibility of the 6-benzylthio group movement to fit better in the hydrophobic pocket at 6-position. This finding prompted us to investigate the influence of sugar-based modification on binding affinity by replacing the oxygen atom from the ribose ring by a methylene group. Therefore, we decided to incorporate the carbasugar moiety, while the hydrophobic 6-benzylthio group was left intact to preserve the selective activity against the parasite and not the host as established in our previous studies.[13,5–21]

The binding affinity (apparent K_m values) of carbocyclic analogue **9a** with an unsubstituted benzylthio group was compared to that of 6-benzylthioinosine as the corresponding ribonucleoside. Compound **9a** exhibited lower binding affinity (105 μM) to the enzyme than that of 6-benzylthioinosine (14.3 μM). It appeared that the carbasugar modification to the ribosyl group is detrimental to

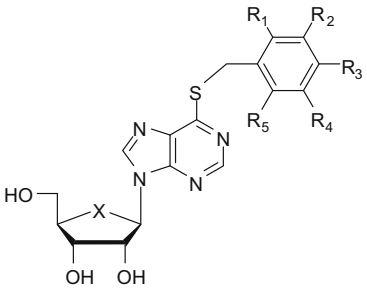


Scheme 1. Synthesis of 6-mercaptopurine derivative **8**. Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C, 1 h, 96%; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C, 1 h, 92%; (c) NaN_3, DMF, 150 °C, 6 h, 85%; (d) H_2, 10% Pd/C, MeOH, 30 psi, 2 h; (e) 5-amino-4,6-dichloropyrimidine, Et_3N, *n*-PrOH, reflux, 24 h, 68% (two steps); (f) $CH(OEt)_3$, *p*-TsOH, rt, 14 h, 65%; (g) thiourea, EtOH, reflux, 3 h; (h) TFA/H_2O (2:1, v/v), 50 °C, 3 h, 52% (two steps).



Scheme 2. Synthesis of carbocyclic 6-benzylthioinosine analogues **9a**–**u**. Reagents and conditions: (a) appropriate benzylhalide, NH_4OH/H_2O, rt, 11 h.

Table 1
Binding affinities (apparent K_m) of carbocyclic 6-benzylthioinosine (**9a**), its analogues (**9b**–**u**) and 6-benzylthioinosine to *Toxoplasma gondii* adenosine kinase



Compound	X	R_1	R_2	R_3	R_4	R_5	K_m[a] (μM)
9a	CH_2	H	H	H	H	H	105.1 ± 12.4
9b	CH_2	F	H	H	H	H	54.8 ± 10.2
9c	CH_2	Cl	H	H	H	H	24.2 ± 5.4
9d	CH_2	CH_3	H	H	H	H	24.9 ± 3.3
9e	CH_2	H	NO_2	H	H	H	26.6 ± 2.7
9f	CH_2	H	CF_3	H	H	H	22.2 ± 1.3
9g	CH_2	H	CH_3	H	H	H	26.8 ± 4.2
9h	CH_2	H	H	F	H	H	31.9 ± 8.3
9i	CH_2	H	H	Cl	H	H	15.6 ± 2.2
9j	CH_2	H	H	Br	H	H	21.1 ± 3.7
9k	CH_2	H	H	NO_2	H	H	94.1 ± 15.2
9l	CH_2	H	H	CN	H	H	128.7 ± 25.0
9m	CH_2	H	H	CO_2CH_3	H	H	40.9 ± 3.2
9n	CH_2	H	H	CH_3	H	H	7.5 ± 0.6
9o	CH_2	H	H	SO_2CH_3	H	H	73.7 ± 7.8
9p	CH_2	H	H	OCF_3	H	H	65.5 ± 7.2
9q	CH_2	H	H	OCH_3	H	H	62.0 ± 4.7
9r	CH_2	Cl	H	Cl	H	H	60.7 ± 7.9
9s	CH_2	H	Cl	Cl	H	H	16.8 ± 4.1
9t	CH_2	F	H	H	H	Cl	31.3 ± 4.2
9u	CH_2	CH_3	H	CH_3	H	CH_3	63.1 ± 6.9
6-Benzyl-thioinosine	O	H	H	H	H	H	14.3 ± 2.4

[a] All values represent the mean ± S.D. of at least two independent experiments with three replica each as previously described.[14–19]

the binding. It can be, therefore, assumed that the oxygen atom of the ribose ring is important, but not critical, for binding to the enzyme. Nevertheless, various substituents appended to the aromatic ring of the benzylthio group of the carbocyclic nucleoside **9a** resulted in the regaining of its binding affinity. All of the carbocyclic nucleoside analogues, with the exception of **9l** (*p*-cyano), showed an increased binding affinity to the enzyme as compared to the unsubstituted compound **9a**. Therefore, these substituents seem to provide additional proper interactions with the surrounding residues in the hydrophobic pocket at the 6-position. Single substitutions at the *ortho* or *meta*-position resulted in an increase in the binding affinity to the enzyme. They were approximately 2–5-fold more potent than the unsubstituted compound **9a**. Interestingly, *meta* substitutions such as *m*-nitro (**9e**, 26.6 μM), *m*-trifluoromethyl (**9f**, 22.2 μM), and *m*-methyl (**9g**, 26.8 μM) exhibited almost similar binding affinities. Therefore, it appears that there is no difference in the binding affinity between electron-withdrawing and electron-donating substituents at the *meta*-position. Apparently, the nature of the substituent at the *para*-position has a substantial impact on the binding affinity to the enzyme. Carbocyclic 6-(*p*-methylbenzylthio)inosine (**9n**, 7.5 μM) was the best ligand displaying a 14-fold increase in the binding affinity relative to the unsubstituted compound **9a** (105 μM). On the other hand, introduction of cyano substituent at *para*-position resulted in a decreased binding affinity, **9l** (*p*-cyano, 129 μM), which was the weakest ligand in this carbocyclic series. In the case of di-substituted carbocyclic analogues **9r**–**t**, *meta*–*para* di-substitutions (**9s**, 16.8 μM) resulted in an increase in the binding affinity relative to *ortho*–*meta* di-substitutions (**9r**, 60.7 μM) or di-*ortho* substitutions (**9t**, 31.3 μM). However, trisubstituted methyl group (**9u**, *o*,*m*,*p*-trimethyl, 63.1 μM) showed decreased binding affinity in comparison to single methyl substitution at the *ortho* (**9d**, 24.9 μM), *meta* (**9g**, 26.8 μM) or *para* (**9n**, 7.5 μM) position.

These results illustrate that the nature and position of the substituent(s) on the aromatic ring of the benzylthio group seem to have distinct influences on the binding affinity of carbocyclic 6-benzylthioinosine (Table 1). These results revealed a different pattern of binding than that of 6-benzylthioinosine analogues.[15,16] Previous structure–activity relationships studies on 6-benzylthioinosines[14–16] and 7-deaza-6-benzylthioinosines[18,19] indicated that compounds having the *p*-cyano substituent were among the best ligands of *T. gondii* adenosine kinase while the carbocyclic nucleoside with same substituent (carbocyclic 6-(*p*-cyanobenzylthio)inosine **9l** had the weakest binding affinity. These results indicate that the binding affinity for *T. gondii* adenosine kinase is sensitive to the sugar motif conformational distribution at the enzyme binding site. It is possible that the carbasugar moiety slightly changed the overall orientation of these analogues inside the binding site and thus carbocyclic nucleosides with the functionalized aromatic group are recognized by the enzyme active site in a slightly different manner than do the corresponding ribonucleosides.

2.3. Evaluation of anti-toxoplasma activity

To determine whether these carbocyclic 6-benzylthioinosines retain the anti-toxoplasmic activities of the 6-benzylthioinosines,[15,16,20,21] the two best ligands, carbocyclic 6-(*p*-methylbenzylthio)inosine (**9n**, 7.5 μM) and carbocyclic 6-(*p*-chlorolbenzylthio)inosine (**9i**, 15.6 μM) (Table 1), were tested for their anti-toxoplasma efficacy and host-toxicity in tissue culture. Pyrimethamine and sulfadiazine as chemotherapeutic agents for the treatment of toxoplasmosis were used as references. As shown in Table 2 the two analogues were effective against infection with the wild type (RH) *T. gondii* in a dose-dependent manner, and neither of the two

analogues was found effective against infection with adenosine kinase deficient strain (*TgAK*$^{-3}$).[31] In agreement with their binding affinities (Table 1), **9n** and **9i** showed potent cellular activity with IC_{50} values of 12 and 15 μM, respectively. Furthermore, **9n** and **9i** exhibited close to, or better potency than pyrimethamine (IC_{50} = 16 μM) and sulfadiazine (IC_{50} = 27 μM). The lack of activity against infection with the adenosine kinase deficient (*TgAK*^{3}) strain of *T. gondii* demonstrates that these compounds are active substrates for *T. gondii* adenosine kinase in vivo as was as in vitro.

The two carbocyclic 6-benzylthioinosine analogues **9n** and **9i** were also tested for their host-toxicity on human fibroblasts in culture using the MTT assay (Table 2). In this assay, neither of the two compounds showed any substantial effect on the viability of the uninfected host cells at concentrations up to 50 μM. Therefore, these carbocyclic analogues can still exhibit selective toxicity against the parasite and may hold a promise in the development of anti-toxoplasmic agents.

2.4. Molecular modeling

T. gondii adenosine kinase[31] is a key purine metabolic enzyme. The *T. gondii* adenosine kinase is a 363-residue (39.3 kDa) monomeric protein that catalyzes the phosphorylation of adenosine to adenosine 5′-monophosphate (AMP), using ATP as a co-substrate. Recent studies[15,18,32–34] suggested that the enzyme becomes functional through induced fit movement to accommodate various classes of substrates, and leads to effective phosphate transfer catalysis. Various co-crystal structures of *T. gondii* adenosine kinase suggest that substituents at the 6-position of purine nucleoside analogues are accommodated in a hydrophobic pocket of the enzyme. Our previous studies also highlighted the molecular basis for the binding of various 6-substituted inosine analogues in the active site of *T. gondii* adenosine kinase.[15,18] In addition, these previous studies also demonstrated that substituted benzyl moieties at the 6-position of the purine ring provided stronger binding through utilization of the hydrophobic pocket of *T. gondii* adenosine kinase.

In an effort to elucidate the structure–activity relationships observed at the *T. gondii* and guide further SAR studies, molecular docking of the most potent inhibitor 6-(*p*-methylbenzylthio)inosine (**9n**) and least potent 6-(*p*-cyanobenzylthio)inosine (**9l**) into adenosine binding site of *T. gondii* adenosine kinase (1LII.pdb). The binding mode of compounds **9n** and **9l** are depicted in Figure 2a and b, respectively. The presence of hydrophobic methyl group on the 6-benzyl substitution favors the overall interaction (glideXP score, −8.15; total energy, −114.2 kJ/mol; Table 3) and shows that the carbasugar adopted an appropriate conformation. The superimposed structure of the docking pose of 6-benzylthioinosine (Fig. 2c) and carbocyclic 6-benzylthioinosine (**9n**) clearly reveals the adoption of the *anti*-conformation and ring-mimicry. Figure 2c also shows that the carbocyclic analogues can bind to the active site of *T. gondii* adenosine kinase with a 2′-*endo* puckered conformation. Furthermore, 6-benzylthioinosine and the *p*-methylbenzyl moiety of the carbocyclic nucleoside **9n** superimposes well with those of 6-benzylthioinosine. From the docking and energetic results it is observed that the compound **9n** has more favorable energetics for the overall interaction with the enzyme binding site. This is probably due to the presence of the carbocyclic ring, which may provide better conformational flexibility to the OH groups at the 2'- and 3'-postions to interact with neighboring residues such as Asp24, Gly69 and Asn73. Two direct protein–adenine hydrogen bonds are formed between the amide N atom of Ser70 and N3 of the purine ring as well as the O^{γ} of Thr140 and N7 of the purine ring. The purine ring is also stabilized by stacking and van der Waals interactions with the side chain of Try169. Additional interactions between the carbocyclic analogues and the enzyme active site result from the substitutions on the purine moiety. The 6-thiobenzyl group occupies the hydrophobic pocket at the 6-position and is involved in van der Waals interaction with the surrounding residues such as Leu46, Leu142, Phe201 and Tyr206.

The orientation of the 6-benzylthio substitution of compound **9l** was completely different from that of **9n** (Fig. 2b). The distance between the 5'-OH group and the triphosphate chain of the β,γ-methylene adenosine 5′-triphosphate (AMP-PCP, a non-hydrolysable ATP analogue) is critical for the phosphorylation. Compound **9l** looses the hydrogen bonding interaction between its 5'-OH group and the γ-phosphate of AMP-PCP as well as that between the amide N atom of Ser70 and the N3 of the purine. The presence of the *p*-CN group not only changes the favorable orientation of *p*-cyanobenzyl group but also does not favor the overall interaction of 6-(*p*-cyanobenzylthio)inosine (**9l**) with the enzyme binding site (glide XP score −5.17; total energy −46.7 kJ/mol, Table 3) and ultimately leads to the least binding affinity.

3. Conclusion

In conclusion, we have described the synthesis of a series of new carbocyclic 6-benzylthioinosine analogues from D-ribose as potent and selective anti-toxoplasmic subversive substrates. We have successfully used parallel solution phase synthesis in incorporation of the substituted benzyl group, which allowed the rapid synthesis and examination of structure–activity relationships of various carbocyclic 6-benzylthioinosines. The present experimental investigations and theoretical calculations revealed that the

Table 2
The effect of carbocyclic 6-(*p*-methylbenzylthio)inosine (**9n**), carbocyclic 6-(*p*-chlorolbenzylthio)inosine (**9i**), and therapeutic compounds on host-toxicity[a] and percent survival[b] of wild type (RH) and adenosine kinase deficient (*TgAK*$^{-3}$) strains of *Toxoplasma gondii* grown in human fibroblasts in culture

Compound	Infection	Concentration (μM)					IC_{50} (μM)
		0	5	10	25	50	
9n (*p*-CH_3)	Wild type (RH)	100	43	2.5	0	0	11.9 ± 0.4
	TgAK^{-3}	100	99	100	100	100	
	None	100	100	100	100	100	
9i (*p*-Cl)	Wild type (RH)	100	73	12	0	0	14.5 ± 1.3
	TgAK^{-3}	100	100	100	100	100	
	None	100	100	100	100	100	
Pyrimethamine[c]	Wild type (RH)	100	99	55	25	23	16.1 ± 2.5
	None	100	101	100	108	108	
Sulfadiazine[a]	Wild type (RH)	100	93	58	53	46	27.3 ± 3.3
	None	100	98	100	100	102	

[a] Host-toxicity of uninfected cells was measured by MTT method in at least two independent experiments each of three replica as previously described.[15–21]
[b] Percent survival of parasites was measured by incorporation of [5,6-^{13}H]uracil in at least two independent experiments of three replica each as previously described.[15–21]
[c] Therapeutic compounds.

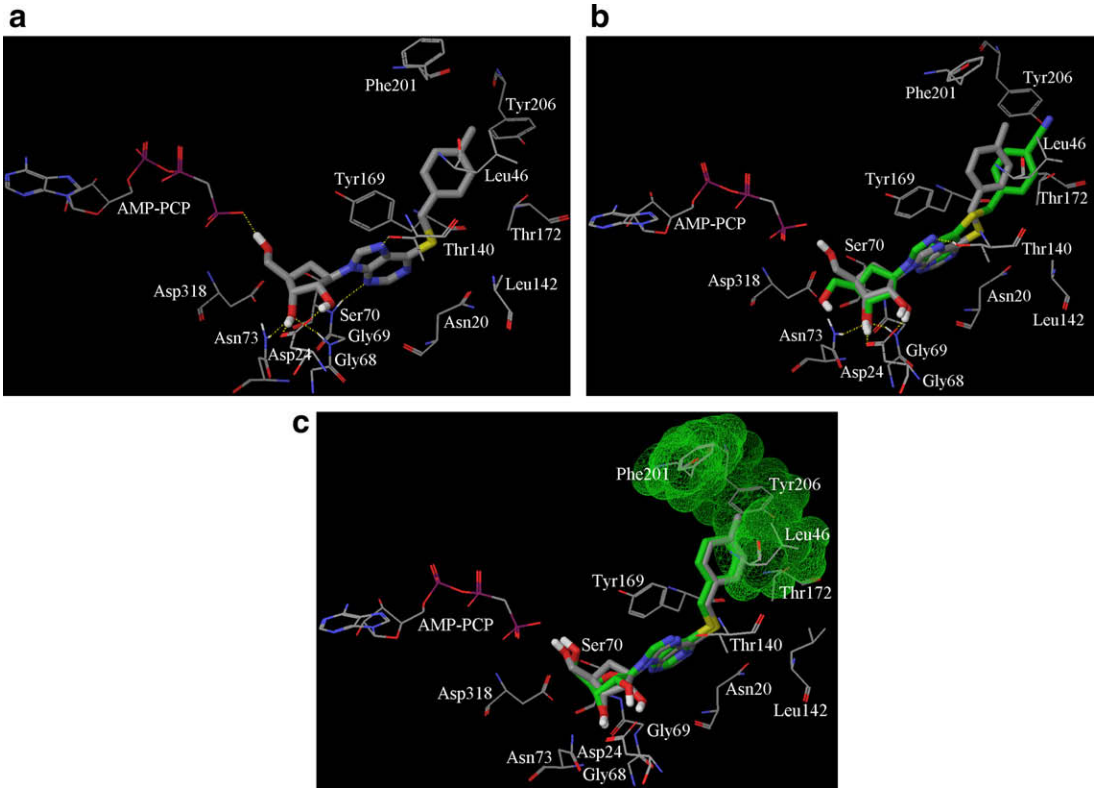


Figure 2. (a) Binding mode of carbocyclic 6-(*p*-methylbenzylthio)inosine (**9n**, carbon color gray) showing the polar interaction as well as orientation of the methyl group, which is more towards the hydrophobic pocket. (b) Superimposed structure of the most active substrate carbocyclic 6-(*p*-methylbenzylthio)inosine (**9n**, carbon color gray) and the least active carbocyclic 6-(*p*-cyanobenzylthio)inosine (**9l**, carbon color green) showing difference in the orientation of the *p*-methylbenzyl and *p*-cyanobenzyl group inside the binding pocket. H-atoms are not shown for the sake of clarity. (c) Superimposed structure of the carbocyclic 6-(*p*-methylbenzylthio)inosine (**9n**, carbon color gray) and 6-benzylthioinosine (carbon color green) showing the same binding mode with the nearby residue in the active site with hydrophobic surface area of Leu46, Phe201 and Tyr206.

Table 3
XP GScore and MBAE (multi-ligand bimolecular association with energetics) calculation of carbocyclic 6-benzylthioinosine analogues after Glide XP docking and energy minimization

Compound	K_m (μM)	XP GScore	Energy difference results (ΔE, kJ/mol)		
			Total energy	vdW[a]	Electrostatic
9l (*p*-CN)	128.7 ± 25.0	−5.17	−46.65	−41.77	−275.90
9n (*p*-CH_3)	7.5 ± 0.6	−8.15	−114.17	−10.23	−425.68

[a] van der Waals interaction.

oxygen atom in sugar moiety is important, but not critical, for the binding to the enzyme because of the preference of the enzyme for ribose rather than carbasugar ring in the pentose binding region. The replacement of the ribose ring of the parent compound 6-benzylthioinosine with a carbasugar moiety resulted in appreciable changes in binding affinities. Nevertheless, there is significant conformational similarity between carbocyclic 6-benzylthioinosine and the parent compound 6-benzylthioinosine. The molecular docking studies demonstrated the adaptation of an *anti*-conformation and overall ligand-fitting into the active site of *T. gondii* adenosine kinase by carbocyclic nucleosides. The extended functionality of the aromatic ring of the carbocyclic nucleosides by substituents such as *p*-methyl led to improvement in the binding affinity. Among the synthesized analogues, carbocyclic 6-(*p*-methylbenzylthio)inosine (**9n**) was the best ligand and also showed high potency in terms of the high anti-toxoplasmic efficacy and lack of host-toxicity in cell-based studies. These observations could provide useful guidance towards the development of novel carbocyclic nucleosides as potential anti-toxoplasmic agents.

4. Experimental section

4.1. Chemistry: general procedures

[8-^{14}C]Adenosine (55 Ci/mol) and [5,6-^{13}H]uracil were purchased from Moravek Biochemicals. RPMI-1640 medium from GIBCO BRL; fetal bovine serum (FBS) from HyClone Laboratories. All other chemicals and compounds were obtained from Sigma Chemical Co. or Fisher Scientific. Melting points were determined on a Mel-temp II apparatus and are uncorrected. Optical rotation was determined on a Jasco DIP-370 Digital Polarimeter. UV spectra were obtained on a Beckman DU-650 spectrophotometer. High resolution mass spectra were recorded on an Agilent 1100 series LC with Waters LCT Premier™. NMR spectra were recorded on a Varian Inova 500 spectrometer and chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane as internal reference. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quarter), or m (multiplet). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Silica gel 60 (220–440 mesh) was used for flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

4.1.1. (1*S*,2*S*,3*R*,4*R*)-4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy)cyclopentan-1-ol (3)

A solution of compound **2** (1.00 g, 4.13 mmol) in MeOH (10.3 mL) was treated with sodium borohydride (0.312 g, 8.25 mmol) at 0 °C and then stirred for 1 h. After the reaction mixture was quenched with water, it was diluted with EtOAc and then washed with brine. The organic layer was dried over Na_2SO_4 and concentrated to

dryness in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 9:1) to give **3** (0.970 g, 96%) as an oil: $[\alpha]_D^{26} = -16.67$ (*c* 0.96, $CHCl_3$) [lit.[24] $[\alpha]_D^{26} = -16.95$ (*c* 1.59, $CHCl_3$)]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.14 (s, 9H), 1.36 (s, 3H), 1.49 (s, 3H), 1.84 (m, 2H), 2.21 (m, 1H), 2.43 (d, J = 8.5 Hz, 1H), 3.21 (dd, J_1 = 9.0 Hz, J_2 = 4.5 Hz, 1H), 3.32 (dd, J_1 = 8.5 Hz, J_2 = 5.0 Hz, 1H), 4.24 (m, 1H), 4.45 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.2, 26.1, 27.3, 35.7, 42.1, 63.0, 72.0, 72.5, 79.6, 83.4, 110.5.

4.1.2. (1*S*,2*S*,3*R*,4*R*)-4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy)-1-[(methylsulfonyl)oxy]-cyclopentane (4)

A solution of compound **3** (3.24 g, 13.3 mmol) in CH_2Cl_2 (66.0 mL) was treated with methanesulfonyl chloride (1.1 mL, 13.7 mmol) and Et_3N (2.8 mL, 19.9 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then washed with brine. The organic layer was dried over Na_2SO_4, and concentrated to dryness in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 8:1) to give **4** (3.94 g, 92%) as an oil: $[\alpha]_D^{27} = -59.49$ (*c* 1.13, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.13 (s, 9H), 1.31 (s, 3H), 1.47 (s, 3H), 1.93 (m, 1H), 2.24 (m, 2H), 3.05 (s, 3H), 3.24 (m, 1H), 3.35 (m, 1H), 4.43 (d, J = 5.5 Hz, 1H), 4.59 (t, J = 5.5 Hz, 1H), 5.11 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.5, 26.2, 27.3, 32.8, 38.6, 41.6, 63.1, 72.9, 78.7, 80.0, 83.5, 111.2.

4.1.3. (1*R*,2*S*,3*R*,4*R*)-1-Azido-4-(*tert*-butoxymethyl)-2,3-(isopropylidenedioxy) cyclopentane (5)

A solution of compound **4** (3.94 g, 12.2 mmol) and sodium azide (7.93 g, 122 mmol) in DMF (122.0 mL) was heated at 150 °C for 6 h. Then it was diluted with water and extracted with EtOAc. The combined organic layer was washed with brine and dried over Na_2SO_4. After the solvent was removed in vacuo, the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 9:1) to give **5** (2.79 g, 85%) as an oil: $[\alpha]_D^{27}$ = -48.95 (*c* 0.67, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.18 (s, 9H), 1.30 (s, 3H), 1.46 (s, 3H), 1.71 (m, 1H), 2.27 (m, 2H), 3.29 (m, 1H), 3.38 (m, 1H), 3.95 (m, 1H),), 4.40 (dd, J_1 = 6.5 Hz, J_2 = 2.0 Hz, 1H), 4.47 (dd, J_1 = 6.5 Hz, J_2 = 2.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.5, 26.9, 27.5, 31.7, 45.4, 62.2, 67.0, 72.7, 82.6, 85.1, 111.3.

4.1.4. (1′*R*,2′*S*,3′*R*,4′*R*)-1-(5-Amino-6-chloropyrimidylamino)-4-(*tert*-butoxymethyl)-2,3-(isopropyl-idenedioxy)cyclopentane (6)

A solution of compound **5** (1.00 g, 4.13 mmol) in MeOH (10.3 mL) was shaken under 30 psi of a hydrogen atmosphere in the presence of 10% Pd/C (300 mg) for 2 h. The reaction mixture was filtered through a pad of celite and then concentrated to dryness in vacuo. The residue was dried and used next reaction without further purification. The crude product in *n*-PrOH (20.7 mL) was treated with 5-amino-4,6-dichloropyrimidine (0.745 g, 4.54 mmol) and Et_3N (2.9 mL, 20.7 mmol). The reaction mixture was heated to reflux for 24 h. After the solvent was removed in vacuo, the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 95:5) to give **6** (1.04 g, 68% for two steps) as an oil: $[\alpha]_D^{27} = -47.85$ (*c* 0.81, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22 (s, 9H), 1.28 (s, 3H), 1.48 (s, 3H), 1.53 (m, 1H), 2.41 (m, 1H), 2.62 (m, 1H), 3.42 (dd, J_1 = 9.5 Hz, J_2 = 3.5 Hz, 1H), 3.51 (m, 3H), 4.41 (m, 1H), 4.48 (m, 1H), 4.54 (m, 1H), 5.84 (d, J = 8.5 Hz, 1H), 8.06 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.6, 27.1, 27.6, 34.2, 45.2, 58.0, 63.4, 73.8, 83.7, 87.3, 110.7, 122.4, 141.9, 149.3, 154.1; HRMS $[M+H]^+$ *m/z* calcd for $C_{17}H_{28}ClN_4O_3$ 371.1850, found 371.1874.

4.1.5. (1′*R*,2′*S*,3′*R*,4′*R*)-9-[4-(*tert*-Butoxymethyl)-2,3-(isopropyl-idenedioxy)cyclopentan-1-yl]-6-chloropurine (7)

A solution of compound **6** (0.588 g, 1.59 mmol) and *p*-toluenesulfonic acid momohydrate (15.1 mg, 0.0793 mmol) in ethyl orthoformate (5.3 mL) was stirred for 14 h at room temperature and then concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4:1) to give **7** (0.394 g, 65%) as an oil: $[\alpha]_D^{27} = -41.92$ (*c* 0.64, $CHCl_3$); UV (MeOH) λ_{max} 264.0 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.23 (s, 9H), 1.35 (s, 3H), 1.61 (s, 3H), 2.41 (m, 1H), 2.51 (m, 1H), 2.63 (m, 1H), 3.52 (m, 1H), 3.57 (m, 1H), 4.66 (m, 1H), 4.94 (m, 1H), 4.99 (m, 1H), 8.31 (s, 1H), 8.78 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.2, 27.5, 27.6, 33.8, 43.8, 62.1, 62.4, 73.0, 81.7, 84.3, 113.4, 118.5, 132.1, 144.3, 151.0, 151.7; HRMS $[M+H]^+$ *m/z* calcd for $C_{18}H_{26}ClN_4O_3$ 381.1693, found 381.1693.

4.1.6. (1′*R*,2′*S*,3′*R*,4′*R*)-9-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-mercaptopurine (8)

A solution of compound **7** (1.01 g, 2.65 mmol) and thiourea (0.404 g, 5.30 mmol) in EtOH (13.0 mL) was heated to reflux for 3 h. The reaction mixture was concentrated to dryness and the residue was purified by short column chromatography on silica gel (CH_2Cl_2/MeOH = 95:5) to give a solid. The resultant compound was dissolved in 18.0 mL of trifluoroacetic acid/H_2O (2:1, v/v) and heated at 50 °C for 3 h. After evaporation of the solvent in vacuo, the residue was triturated with EtOH and the resulting solid was filtered and dried to give **8** (0.389 g, 52% for two steps) as a white solid: mp 260–262 °C (dec); $[\alpha]_D^{24} = -63.39$ (*c* 0.37, DMSO); UV (H_2O) λ_{max} 321.0 nm; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.66 (m, 1H), 2.01 (m, 1H), 2.22 (m, 1H), 3.44 (m, 2H), 3.80 (m, 1H), 4.25 (m, 1H), 4.70 (m, 1H), 8.16 (s, 1H), 8.38 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 29.9, 45.8, 59.8, 63.4, 72.0, 75.4, 135.9, 142.5, 145.0, 176.2; HRMS $[M+H]^+$ *m/z* calcd for $C_{11}H_{15}N_4O_3S$ 283.0865, found 283.0816.

4.1.7. General procedure for the synthesis of carbocyclic nucleoside analogues (9a–u)

Compound **8** (60.0 mg, 0.213 mmol) was added to each reaction vessel of Argonaut Quest 210 Organic Synthesizer and then H_2O (4.0 mL) and concentrated NH_4OH (0.02 mL) were added. To this solution, the appropriate benzylbromides (1.3 equiv, 0.266 mmol) were added. The reaction mixtures were stirred vigorously (upward stroke = 50%, time = 4 s) for 11 h at room temperature. The reaction vessels were drained and the collected crude material was co-evaporated with ethanol in vacuo. The residues were purified by short column chromatography on silica gel (CH_2Cl_2/MeOH = 95:5) to give the desired products as white solids.

4.1.7.1. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)cyclopentan-1-yl]-6-benzylthiopurine (9a). Yield 90%; mp 110–111 °C; $[\alpha]_D^{27} = -48.32$ (*c* 0.27, MeOH); UV (H_2O) λ_{max} 293.0 nm (ε 18,100, pH 2), 293.0 nm (ε 18,600, pH 7), 293.0 nm (ε 18,700, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.28 (m, 1H), 2.49 (m, 1H), 3.73 (m, 2H), 4.08 (m, 1H), 4.60 (m, 1H), 4.71 (s, 2H), 4.96 (m, 1H), 7.27 (m, 1H), 7.32 (m, 2H), 7.49 (d, J = 7.0 Hz, 2H), 8.46 (s, 1H), 8.75 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 32.1, 45.5, 60.7, 63.3, 72.3, 75.0, 127.0, 128.2, 128.8, 130.9, 137.6, 143.5, 148.6, 151.2, 160.1. Anal. Calcd for $C_{18}H_{20}N_4O_3S{\cdot}0.3H_2O$: C, 57.22; H, 5.50; N, 14.83; S, 8.49. Found: C, 56.96; H, 5.65; N, 14.75; S, 8.42.

4.1.7.2. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(2-fluorobenzyl-thio)purine (9b). Yield 94%; mp 104–106 °C; $[\alpha]_D^{27} = -43.08$ (*c* 0.30, MeOH); UV (H_2O) λ_{max} 292.0 nm (ε 21,000, pH 2), 291.0 nm (ε 21,100, pH 7), 291.0 nm (ε 21,100, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.29 (m, 1H), 2.49 (m, 1H), 3.74 (m, 2H), 4.08 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.75 (s, 2H), 4.96 (m, 1H), 7.13 (m, 2H), 7.31 (m, 1H), 7.60 (t, J = 7.5 Hz, 1H), 8.46 (s, 1H), 8.76 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 25.3, 28.6, 45.5, 60.7, 63.3, 72.3, 74.9, 114.9 (d, J = 21.4 Hz), 124.0, 124.7 (d, J = 14.4 Hz), 129.1 (d, J = 8.1 Hz), 130.9, 131.1, 143.6, 148.7, 151.1, 159.6, 161.1 (d, J = 245.1 Hz).

Anal. Calcd for $C_{18}H_{19}FN_4O_3S{\cdot}0.2H_2O$: C, 54.87; H, 4.96; N, 14.22; S, 8.14. Found: C, 54.78; H, 5.01; N, 14.12; S, 8.03.

4.1.7.3. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)cyclopentan-1-yl]-6-(2-chlorobenzyl-thio)purine (9c). Yield 90%; mp 100–101 °C; $[\alpha]_D^{28}$ = −42.09 (*c* 0.14, MeOH); UV (H_2O) λ_{max} 292.0 nm (ε 21,600, pH 2), 292.0 nm (ε 21,400, pH 7), 292.0 nm (ε 21,400, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.28 (m, 1H), 2.49 (m, 1H), 3.74 (m, 2H), 4.08 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.84 (s, 2H), 4.97 (m, 1H), 7.28 (m, 2H), 7.45 (m, 1H), 7.69 (m, 1H), 8.46 (s, 1H), 8.77 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 29.9, 45.5, 60.7, 63.3, 72.3, 75.0, 126.8, 128.8, 129.2, 130.9, 131.1, 134.0, 135.3, 143.6, 148.6, 151.1, 159.7. Anal. Calcd for $C_{18}H_{19}ClN_4O_3S$: C, 53.13; H, 4.71; N, 13.77; S, 7.88. Found: C, 52.84; H, 4.86; N, 13.56; S, 7.74.

4.1.7.4. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(2-methylbenzyl-thio)purine (9d). Yield 91%; mp 92–93 °C; $[\alpha]_D^{28}$ = -45.57 (*c* 0.14, MeOH); UV (H_2O) λ_{max} 294.0 nm (ε 21,800, pH 2), 293.0 nm (ε 21,900, pH 7), 293.0 nm (ε 21,900, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.05 (m, 1H), 2.29 (m, 1H), 2.47 (s, 3H), 2.51 (m, 1H), 3.74 (m, 2H), 4.09 (m, 1H), 4.61 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.73 (s, 2H), 4.96 (m, 1H), 7.19 (m, 3H), 7.46 (d, *J* = 8.0 Hz, 1H), 8.46 (s, 1H), 8.77 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 18.1, 28.6, 30.6, 45.5, 60.7, 63.3, 72.3, 75.0, 125.8, 127.5, 129.8, 130.1, 130.8, 134.7, 136.8, 143.5, 148.5, 151.2, 160.3. Anal. Calcd for $C_{19}H_{22}N_4O_3S{\cdot}0.2H_2O$: C, 58.50; H, 5.79; N, 14.36; S, 8.22. Found: C, 58.36; H, 5.98; N, 14.27; S, 8.23.

4.1.7.5. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(3-nitrobenzyl-thio)purine (9e). Yield 88%; mp 84–86 °C; $[\alpha]_D^{27}$ = −39.93 (*c* 0.24, MeOH); UV (H_2O) λ_{max} 287.0 nm (ε 29,900, pH 2), 287.0 nm (ε 11,100, pH 7), 286.0 nm (ε 9900, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.28 (m, 1H), 2.49 (m, 1H), 3.73 (m, 2H), 4.08 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.81 (s, 2H), 4.97 (m, 1H), 7.57 (t, *J* = 8.0 Hz, 1H), 7.94 (d, *J* = 7.0 Hz, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 8.43 (s, 1H), 8.48 (s, 1H), 8.75 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.5, 31.0, 45.5, 60.7, 63.3, 72.2, 74.9, 121.7, 123.6, 129.3, 131.0, 135.1, 140.9, 143.8, 148.2, 148.7, 151.2, 159.0. Anal. Calcd for $C_{18}H_{19}N_5O_5S$: C, 51.79; H, 4.59; N, 16.78; S, 7.68. Found: C, 51.61; H, 4.77; N, 16.58; S, 7.67.

4.1.7.6. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(3-trifluoro-methylbenzylthio)purine (9f). Yield 87%; mp 118–119 °C; $[\alpha]_D^{27}$ = −42.20 (*c* 0.33, MeOH); UV (H_2O) λ_{max} 292.0 nm (ε 21,000, pH 2), 292.0 nm (ε 20,500, pH 7), 292.0 nm (ε 20,500, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.28 (m, 1H), 2.49 (m, 1H), 3.73 (m, 2H), 4.08 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.78 (s, 2H), 4.96 (m, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.56 (d, *J* = 7.5 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.84 (s, 1H), 8.47 (s, 1H), 8.75 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.5, 31.3, 45.5, 60.7, 63.3, 72.2, 74.9, 123.6, 125.5, 128.9, 130.3 (q, *J* = 32.0 Hz), 131.0, 132.6, 139.7, 143.7, 148.7, 151.1, 159.3. Anal. Calcd for $C_{19}H_{19}F_3N_4O_3S{\cdot}0.1H_2O$: C, 51.60; H, 4.38; N, 12.67; S, 7.25. Found: C, 51.45; H, 4.41; N, 12.57; S, 7.18.

4.1.7.7. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(3-methylbenzyl-thio)purine (9g). Yield 89%; mp 112–113 °C; $[\alpha]_D^{28}$ = −38.92 (*c* 0.16, MeOH); UV (H_2O) λ_{max} 294.0 nm (ε 22,500, pH 2), 293.0 nm (ε 22,400, pH 7), 293.0 nm (ε 22,200, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.29 (m, 1H), 2.33 (s, 3H), 2.49 (m, 1H), 3.74 (m, 2H), 4.09 (m, 1H), 4.60 (dd, J_1 = 9.0 Hz, J_2 = 5.5 Hz, 1H), 4.66 (s, 2H), 4.96 (m, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 7.20 (t, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.5 Hz, 1H), 7.30 (s, 1H), 8.46 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 20.1, 28.6, 32.2, 45.5, 60.7, 63.3, 72.3, 75.0, 125.9, 127.7, 128.1, 129.4, 130.8, 137.3, 138.0, 143.4, 148.5, 151.2, 160.2. Anal. Calcd for $C_{19}H_{22}N_4O_3S{\cdot}0.1H_2O$: C, 58.77; H, 5.76; N, 14.43; S, 8.26. Found: C, 58.56; H, 5.83; N, 14.30; S, 8.23.

4.1.7.8. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)cyclopentan-1-yl]-6-(4-fluorobenzyl-thio)purine (9h). Yield 89%; mp 114–116 °C; $[\alpha]_D^{27}$ = −35.71 (*c* 0.21, MeOH); UV (H_2O) λ_{max} 293.0 nm (ε 20,200, pH 2), 293.0 nm (ε 20,200, pH 7), 292.0 nm (ε 19,700, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.29 (m, 1H), 2.49 (m, 1H), 3.74 (m, 2H), 4.09 (m, 1H), 4.60 (dd, J_1 = 9.0 Hz, J_2 = 5.5 Hz, 1H), 4.69 (s, 2H), 4.96 (m, 1H), 7.05 (t, *J* = 8.5 Hz, 2H), 7.52 (m, 2H), 8.46 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 31.2, 45.5, 60.7, 63.3, 72.3, 74.9, 114.8 (d, *J* = 21.5 Hz), 130.7 (d, *J* = 8.1 Hz), 130.9, 133.9, 143.5, 148.6, 151.2, 159.8, 162.1 (d, *J* = 243.3 Hz). Anal. Calcd for $C_{18}H_{19}FN_4O_3S{\cdot}0.2H_2O$: C, 54.87; H, 4.96; N, 14.22; S, 8.14. Found: C, 54.72; H, 4.98; N, 14.12; S, 7.99.

4.1.7.9. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)cyclopentan-1-yl]-6-(4-chlorobenzyl-thio)purine (9i). Yield 90%; mp 107–108 °C; $[\alpha]_D^{27}$ = −44.44 (*c* 0.34, MeOH); UV (H_2O) λ_{max} 293.0 nm (ε 21,700, pH 2), 293.0 nm (ε 21,800, pH 7), 293.0 nm (ε 21,300, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.29 (m, 1H), 2.49 (m, 1H), 3.73 (m, 2H), 4.08 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.68 (s, 2H), 4.96 (m, 1H), 7.31 (d, *J* = 7.0 Hz, 2H), 7.49 (d, *J* = 7.5 Hz, 2H), 8.46 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 31.2, 45.5, 60.7, 63.3, 72.3, 74.9, 128.2, 130.4, 130.9, 132.7, 136.9, 143.6, 148.6, 151.2, 159.6. Anal. Calcd for $C_{18}H_{19}ClN_4O_3S$: C, 53.13; H, 4.71; N, 13.77; S, 7.88. Found: C, 52.99; H, 4.79; N, 13.61; S, 7.67.

4.1.7.10. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)cyclopentan-1-yl]-6-(4-bromobenzyl-thio)purine (9j). Yield 92%; mp 152–153 °C; $[\alpha]_D^{27}$ = −36.57 (*c* 0.17, MeOH); UV (H_2O) λ_{max} 293.0 nm (ε 21,500, pH 2), 293.0 nm (ε 21,200, pH 7), 293.0 nm (ε 21,400, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.28 (m, 1H), 2.49 (m, 1H), 3.73 (m, 2H), 4.08 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.67 (s, 2H), 4.96 (m, 1H), 7.45 (m, 4H), 8.47 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 31.3, 45.5, 60.7, 63.3, 72.3, 75.0, 120.6, 130.7, 130.9, 131.2, 137.3, 143.6, 148.6, 151.2, 159.6. Anal. Calcd for $C_{18}H_{19}BrN_4O_3S{\cdot}0.2H_2O$: C, 47.52; H, 4.30; N, 12.32; S, 7.05. Found: C, 47.32; H, 4.26; N, 12.13; S, 6.87.

4.1.7.11. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)cyclopentan-1-yl]-6-(4-nitrobenzyl-thio)purine (9k). Yield 87%; mp 89–90 °C; $[\alpha]_D^{27}$ = −46.89 (*c* 0.31, MeOH); UV (H_2O) λ_{max} 288.0 nm (ε 27,300, pH 2), 288.0 nm (ε 27,500, pH 7), 288.0 nm (ε 27,300, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.03 (m, 1H), 2.28 (m, 1H), 2.48 (m, 1H), 3.73 (m, 2H), 4.08 (m, 1H), 4.59 (dd, J_1 = 9.0 Hz, J_2 = 5.0 Hz, 1H), 4.81 (s, 2H), 4.96 (m, 1H), 7.76 (d, *J* = 8.5 Hz, 2H), 8.19 (d, *J* = 8.5 Hz, 2H), 8.48 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 31.0, 45.5, 60.7, 63.2, 72.2, 74.9, 123.1, 129.9, 131.0, 143.8, 146.3, 147.1, 148.8, 151.2, 158.9. Anal. Calcd for $C_{18}H_{19}N_5O_5S$: C, 51.79; H, 4.59; N, 16.78; S, 7.68. Found: C, 51.73; H, 4.72; N, 16.66; S, 7.60.

4.1.7.12. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(4-cyanobenzyl-thio)purine (9l). Yield 92%; mp 114–116 °C; $[\alpha]_D^{24}$ = −37.61 (*c* 0.29, MeOH); UV (H_2O) λ_{max} 292.0 nm (ε 21,500, pH 2), 292.0 nm (ε 21,200, pH 7), 292.0 nm (ε 21,400, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.03 (m, 1H), 2.28 (m, 1H), 2.49 (m, 1H), 3.73 (m, 2H), 4.08 (m, 1H), 4.60 (dd,

J_1 = 8.5 Hz, J_2 = 6.0 Hz, 1H), 4.76 (s, 2H), 4.96 (m, 1H), 7.69 (m, 4H), 8.48 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.5, 31.3, 45.5, 60.7, 63.2, 72.2, 74.9, 110.6, 118.2, 129.8, 131.0, 132.0, 143.7, 144.3, 148.7, 151.1, 159.0. Anal. Calcd for $C_{19}H_{19}N_5O_3S{\cdot}0.6H_2O$: C, 55.90; H, 4.99; N, 17.15; S, 7.85. Found: C, 55.69; H, 4.84; N, 16.84; S, 7.70.

4.1.7.13. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(4-methoxy-carbonylbenzylthio)purine (9m). Yield 91%; mp 126–127 °C; $[\alpha]_D^{25}$ = −38.23 (*c* 0.22, MeOH); UV (H_2O) λ_{max} 293.0 nm (ε 23,200, pH 2), 292.0 nm (ε 22,700, pH 7), 293.0 nm (ε 20,900, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.28 (m, 1H), 2.49 (m, 1H), 3.73 (m, 2H), 3.91 (s, 3H), 4.08 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.76 (s, 2H), 4.96 (m, 1H), 7.62 (d, *J* = 8.5 Hz, 2H), 7.97 (d, *J* = 8.0 Hz, 2H), 8.47 (s, 1H), 8.75 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 31.5, 45.5, 51.2, 60.7, 63.3, 72.2, 74.9, 128.8, 129.0, 129.3, 130.9, 143.7, 143.8, 148.7, 151.2, 159.4, 166.9. Anal. Calcd for $C_{20}H_{22}N_4O_5S{\cdot}0.3H_2O$: C, 55.11; H, 5.23; N, 12.85; S, 7.36. Found: C, 55.02; H, 4.97; N, 12.70; S, 7.19.

4.1.7.14. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(4-methylbenzyl-thio)purine (9n). Yield 91%; mp 112–113 °C; $[\alpha]_D^{27}$ = −41.04 (*c* 0.27, MeOH); UV (H_2O) λ_{max} 294.0 nm (ε 23,200, pH 2), 293.0 nm (ε 23,200, pH 7), 294.0 nm (ε 23,100, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.29 (m, 1H), 2.33 (s, 3H), 2.49 (m, 1H), 3.74 (m, 2H), 4.09 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.65 (s, 2H), 4.96 (m, 1H), 7.13 (d, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 7.5 Hz, 2H), 8.45 (s, 1H), 8.73 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 19.8, 28.6, 32.0, 45.5, 60.7, 63.3, 72.3, 75.0, 128.7, 128.8, 130.8, 134.4, 136.8, 143.4, 148.6, 151.2, 160.3. Anal. Calcd for $C_{19}H_{22}N_4O_3S{\cdot}0.2H_2O$: C, 58.50; H, 5.79; N, 14.36; S, 8.22. Found: C, 58.45; H, 5.74; N, 14.19; S, 8.07.

4.1.7.15. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(4-methyl-sulfonylbenzylthio)purine (9o). Yield 88%; mp 100–102 °C; $[\alpha]_D^{25}$ = −38.80 (*c* 0.45, MeOH); UV (H_2O) λ_{max} 291.0 nm (ε 18,800, pH 2), 291.0 nm (ε 18,900, pH 7), 291.0 nm (ε 18,900, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.29 (m, 1H), 2.49 (m, 1H), 3.74 (m, 2H), 4.08 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.75 (s, 2H), 4.96 (m, 1H), 7.13 (m, 2H), 7.31 (m, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 8.46 (s, 1H), 8.76 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 31.2, 43.0, 45.5, 60.7, 63.2, 72.2, 74.9, 127.2, 129.8, 131.0, 139.4, 143.7, 145.0, 148.7, 151.2, 159.0. Anal. Calcd for $C_{19}H_{22}N_4O_5S_2{\cdot}0.3H_2O$: C, 50.05; H, 5.00; N, 12.29; S, 14.07. Found: C, 49.94; H, 4.90; N, 12.20; S, 13.79.

4.1.7.16. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(4-trifluoro-methoxybenzylthio)purine (9p). Yield 90%; mp 150–151 °C; $[\alpha]_D^{27}$ = −40.27 (*c* 0.26, MeOH); UV (H_2O) λ_{max} 293.0 nm (ε 21,200, pH 2), 292.0 nm (ε 21,000, pH 7), 292.0 nm (ε 19,100, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.29 (m, 1H), 2.49 (m, 1H), 3.73 (m, 2H), 4.08 (m, 1H), 4.60 (dd, J_1 = 9.0 Hz, J_2 = 5.5 Hz, 1H), 4.73 (s, 2H), 4.96 (m, 1H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 9.0 Hz, 2H), 8.47 (s, 1H), 8.75 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 31.0, 45.5, 60.7, 63.3, 72.3, 74.9, 120.5 (q, *J* = 254.3 Hz), 120.7, 130.5, 130.9, 137.4, 143.6, 148.2, 148.7, 151.2, 159.5. Anal. Calcd for $C_{19}H_{19}F_3N_4O_4S$: C, 50.00; H, 4.20; N, 12.27; S, 7.03. Found: C, 49.73; H, 4.27; N, 12.20; S, 6.92.

4.1.7.17. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(4-methoxy-benzylthio)purine (9q). Yield 89%; mp 94–95 °C; $[\alpha]_D^{27}$ = −44.09 (*c* 0.33, MeOH); UV (H_2O) λ_{max} 294.0 nm (ε 22,600, pH 2), 294.0 nm (ε 22,800, pH 7), 294.0 nm (ε 22,200, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.29 (m, 1H), 2.49 (m, 1H), 3.74 (m, 2H), 3.80 (s, 3H), 4.09 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.65 (s, 2H), 4.97 (m, 1H), 6.88 (d, *J* = 8.5 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 8.45 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 31.8, 45.5, 54.3, 60.7, 63.3, 72.3, 74.9, 113.6, 129.3, 130.0, 130.9, 143.4, 148.6, 151.2, 159.1, 160.4. Anal. Calcd for $C_{19}H_{22}N_4O_4S$: C, 56.70; H, 5.51; N, 13.92; S, 7.97. Found: C, 56.41; H, 5.69; N, 13.82; S, 7.87.

4.1.7.18. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(2,4-dichloro-benzylthio)purine (9r). Yield 93%; mp 163–164 °C; $[\alpha]_D^{27}$ = −39.33 (*c* 0.25, MeOH); UV (H_2O) λ_{max} 292.0 nm (ε 22,000, pH 2), 292.0 nm (ε 21,500, pH 7), 291.0 nm (ε 17,400, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.29 (m, 1H), 2.49 (m, 1H), 3.73 (m, 2H), 4.08 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.80 (s, 2H), 4.97 (m, 1H), 7.29 (m, 1H), 7.52 (m, 1H), 7.70 (d, *J* = 8.5 Hz, 1H), 8.47 (s, 1H), 8.77 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 29.2, 45.5, 60.7, 63.3, 72.2, 74.9, 127.0, 128.9, 130.9, 132.2, 133.6, 134.5, 134.8, 143.7, 148.7, 151.1, 159.2. Anal. Calcd for $C_{18}H_{18}Cl_2N_4O_3S$: C, 48.99; H, 4.11; N, 12.69; S, 7.27. Found: C, 49.13; H, 4.21; N, 12.75; S, 7.19.

4.1.7.19. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(3,4-dichloro-benzylthio)purine (9s). Yield 93%; mp 160–161 °C; $[\alpha]_D^{27}$ = −39.73 (*c* 0.30, MeOH); UV (H_2O) λ_{max} 292.0 nm (ε 24,400, pH 2), 292.0 nm (ε 24,500, pH 7), 291.0 nm (ε 19,300, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.04 (m, 1H), 2.28 (m, 1H), 2.49 (m, 1H), 3.73 (m, 2H), 4.08 (m, 1H), 4.60 (dd, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 4.69 (s, 2H), 4.96 (m, 1H), 7.46 (m, 2H), 7.70 (s, 1H), 8.48 (s, 1H), 8.76 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.6, 30.6, 45.5, 60.7, 63.3, 72.3, 74.9, 128.7, 130.1, 130.6, 130.8, 130.9, 131.7, 139.2, 143.7, 148.7, 151.1, 159.1. Anal. Calcd for $C_{18}H_{18}Cl_2N_4O_3S$: C, 48.99; H, 4.11; N, 12.69; S, 7.27. Found: C, 48.96; H, 4.32; N, 12.55; S, 7.13.

4.1.7.20. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(2-chloro-6-fluorobenzylthio)purine (9t). Yield 91%; mp 119–120 °C; $[\alpha]_D^{27}$ = −37.92 (*c* 0.25, MeOH); UV (H_2O) λ_{max} 292.0 nm (ε 22,100, pH 2), 292.0 nm (ε 22,000, pH 7), 291.0 nm (ε 15,900, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.06 (m, 1H), 2.29 (m, 1H), 2.50 (m, 1H), 3.74 (m, 2H), 4.09 (m, 1H), 4.61 (dd, J_1 = 9.0 Hz, J_2 = 5.5 Hz, 1H), 4.93 (s, 2H), 4.97 (m, 1H), 7.16 (t, *J* = 8.5 Hz, 1H), 7.36 (m, 2H), 8.47 (s, 1H), 8.79 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 23.6, 28.6, 45.5, 60.7, 63.3, 72.3, 74.9, 114.0 (d, *J* = 22.5 Hz), 122.8 (d, *J* = 18.1 Hz), 125.4, 129.8 (d, *J* = 9.6 Hz), 130.8, 135.5, 143.7, 148.7, 151.2, 159.6, 161.6 (d, *J* = 248.5 Hz). Anal. Calcd for $C_{18}H_{18}ClFN_4O_3S$: C, 50.88; H, 4.27; N, 13.19; S, 7.55. Found: C, 50.82; H, 4.34; N, 12.97; S, 7.33.

4.1.7.21. (1′*R*,2′*S*,3′*R*,4′*R*)-1-[2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentan-1-yl]-6-(2,4,6-trimethyl-benzylthio)purine (9u). Yield 90%; mp 92–94 °C; $[\alpha]_D^{29}$ = −34.98 (*c* 0.17, MeOH); UV (H_2O) λ_{max} 295.0 nm (ε 22,700, pH 2), 294.0 nm (ε 22,600, pH 7), 294.0 nm (ε 22,500, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 2.05 (m, 1H), 2.28 (m, 4H), 2.40 (s, 6H), 2.50 (m, 1H), 3.74 (m, 2H), 4.09 (m, 1H), 4.61 (dd, J_1 = 9.0 Hz, J_2 = 5.5 Hz, 1H), 4.73 (s, 2H), 4.97 (m, 1H), 6.90 (s, 2H), 8.46 (s, 1H), 8.77 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 18.4, 19.7, 27.9, 28.6, 45.5, 60.7, 63.3, 72.3, 75.0, 128.6, 128.7, 130.9, 137.1, 137.2, 143.4, 148.5, 151.3, 161.3. Anal. Calcd for $C_{21}H_{26}N_4O_3S{\cdot}0.4H_2O$: C, 59.81; H, 6.41; N, 13.29; S, 7.60. Found: C, 59.70; H, 6.36; N, 13.22; S, 7.60.

4.2. Molecular modeling

The X-ray crystal structure of the *T. gondii* adenosine kinase complex with adenosine and AMP-PCP (PDB ID: 1LII.pdb),[32,35] were

used as a target for the modeling calculation of compounds **9n** and **9l**. The ligands (carbocyclic nucleoside) were docked with the active site using the 'xtra precision' (XP) GLIDE algorithm.[36] After successful docking, enzyme–ligand complexes were subjected to energy minimization (EM)[38] to obtain the final binding mode. All the calculations were performed on Schrödinger Suite 2007 (Schrödinger Inc.)[37] with GB/SA continuum water solvation model. OPLS_2001 force field was used for 'xtra precision' docking and then the MMFFs force field was implemented in MACROMODEL 9.1[38] for energy minimization.

4.3. Evaluation of the newly designed carbocyclic 6-benzylthioinosine analogues as alternative substrates for purified *T. gondii* adenosine kinase

Enzyme assays were run under conditions where activity was linear with time and enzyme concentration.[14–19] Activity was determined by following the formation of radiolabeled AMP from adenosine. The assay mixture contains 50 mM Tris–Cl, pH 7.4; 2.5 mM ATP, 5 mM $MgCl_2$, 5 mM creatine phosphate, creatine kinase, 5 μM [8-^{14}C]adenosine (55 Ci/mol), 50 μL purified cloned *T. gondii* adenosine kinase, prepared as previously described[39] in a final volume of 100 μL, in the absence or presence of various concentrations of the compound under evaluation. Incubation was carried out at 37 °C and terminated by boiling in a water bath for 2 min, followed by freezing for at least 20 min. Precipitated proteins were removed by centrifugation and 10 μL of the supernatant were spotted on silica gel TLC plates. The TLC plates were developed in a mixture of chloroform/methanol/acetic acid (102:12:6 v/v/v). The R_f values were: adenosine, 0.27 and AMP, 0.17. The amounts of radioactivity in both the substrate and product were calculated on a percentage basis using a computerized Berthold LB-284 Automatic TLC-Linear Analyzers. Apparent K_i values of these analogues were calculated from Dixon plots $1/v$ versus [I] by least-squares fitting by computer programs written by Dr. Naguib as previously described.[14–20] The synthesis of the nucleoside 5′-monophosphates from the tested carbocyclic 6-benzylthioinosine analogues was confirmed by HPLC and NMR analyses as previously described,[15–19] indicating that these compounds are alternative substrates to *T. gondii* adenosine kinase. Since these compounds are alternate substrates of *T. gondii* adenosine kinase, their apparent K_i values are equal to their apparent K_m values[39] as presented in Table 1.

4.4. Evaluation of carbocyclic 6-benzylthioinosine analogues as potential anti-toxoplasmosis agents against tachyzoites in tissue culture

The wild type RH and the adenosine kinase deficient mutant *TgAK*$^{-3}$strains[31] of *T. gondii* were used in these experiments. The adenosine kinase deficient mutant *TgAK*$^{-3}$ was used as a control to verify that the promising drugs were metabolized by adenosine kinase in vivo as was the case in vitro. The effects of carbocyclic analogues as anti-toxoplasmosis agents in tissue culture was measured by their ability to inhibit the replication of intracellular *T. gondii* in tissue culture, using monolayers of human foreskin fibroblasts (CRL-1634, American Type Culture Collection), grown for no more than 20 passages in RPMI-1640 medium.[15–19,21] The viability of intracellular parasites was evaluated by the selective incorporation of radiolabeled uracil into nucleic acids of the parasites at least in triplicates as previously described.[16–21] Briefly, confluent cells (4–5 day incubation) were cultured for 24 h in the 24-well flat bottom microtiter plates ($\sim 5 \times 10^5$/1 mL/well) and incubated at 37 °C in 5% CO_2, 95% air to allow the cells to attach. The medium was then removed and the cells were infected with isolated *T. gondii* in medium with 3% FBS (1 parasite/cell). After 1 h incubation, the cultures were washed with media with 10% FBS to remove extracellular parasites. FBS was maintained at a final concentration of 10%. Compounds were dissolved in 50% ethanol and then added to cultures of the parasite-infected cells to give a final concentration of 0, 5, 10, 25, and 50 μM. The final concentration of ethanol when the compounds were added to the wells was 2.5%. After an additional 18 h incubation the medium was replaced with 1 mL drug free media containing [5,6-^{13}H]uracil (5 μCi/mL) and incubated for another 6 h after which the media was removed. The fibroblasts were then released from the wells by trypsinization with the addition of 200 μL trypsin/EDTA (2.5×) to each well. After 10 min incubation, 1 mL of ice cold 10% trichloroacetic acid (TCA) was added to each well. The plates were then placed on a shaker to insure the detachment of the cells. The suspended contents of each well was filtered through GF/A 2.4 cm glass microfiber filters (Whatman), which were pre-washed each with 1 mL double distilled H_2O and dried. After filtration, the filters were washed with 10 mL methanol, left to dry, then placed in scintillation vials containing 5 mL of Econo-Safe scintillation fluor (Research Products International Corp, Mount Prospect, IL), and radioactivity was counted using an LS5801 Beckman scintillation counter. The effect of the compounds on the growth of the parasite was estimated as percentage reduction in the uptake of radiolabeled uracil by treated parasites as compared to the untreated controls.[14,20–26,40] Radiolabel incorporation closely correlates with parasite growth.[14,20–26,40]

4.5. Host-toxicity of carbocyclic 6-benzylthioinosine analogues

Possible toxicity against the host cells by equivalent doses of the various analogues used in the above experiments was measured, at least in triplicates, using a modification of the Microculture Tetrazolium (MTT) assay on uninfected monolayers of human foreskin fibroblasts (grown for no more than 20 passages) in RPMI-1640 medium.[15–19,21] Briefly, confluent cells were incubated for at least 24 h in 96-well flat bottom microtiterplates ($\sim 10^5$/200 μL/well) at 37 °C in 5% CO_2, 95% air to allow the cells to attach. The medium was then replaced with 200 μL of fresh medium. The appropriate concentration of the compounds was dissolved in 50 μL of medium, and added to each well to give the desired final concentrations. The cultures were then incubated for 48 h after which 50 μL of sterile MTT solution (2 mg/1 mL PBS) was added to each well. MTT solution was sterilized by filtration through 0.22 μm filters (Costar, Cambridge, MA). After 4 h incubation, the medium was removed and 100 μL of dimethylsulfoxide (DMSO) was added to each well and the plates were shaken gently for 2–3 min to dissolve the formed formazan crystals. The absorbance was measured at 540 nm using a computerized microtiterplate reader (Themomax, Molecular Devices).

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References and notes

1. Montoya, J. G.; Liesenfeld, O. *Lancet* **2004**, *363*, 1965.
2. Hill, D.; Dubey, J. P. *Clin. Microbiol. Infect.* **2002**, *8*, 634.
3. Tenter, A. M.; Heckeroth, A. R.; Weiss, L. M. *Int. J. Parasitol.* **2000**, *30*, 1217.
4. Jones, J. L.; Kruszon-Moran, D.; Wilson, M.; McQuillan, G.; Navin, T.; McAuley, J. B. *Am. J. Epidemiol.* **2001**, *154*, 357.
5. Mead, P. S.; Slutsker, L.; Dietz, V.; McCaig, L. F.; Bresee, J. S.; Shapiro, C.; Griffin, P. M.; Tauxe, R. V. *Emerg. Infect. Dis.* **1999**, *5*, 607.
6. Renold, C.; Sugar, A.; Chave, J. P.; Perrin, L.; Delavelle, J.; Pizzolato, G.; Burkhard, P.; Gabriel, V.; Hirschel, B. *Medicine (Baltimore)* **1992**, *71*, 224.
7. Klepser, M. E.; Klepser, T. B. *Drugs* **1997**, *53*, 40.
8. Kravetz, J. D.; Federman, D. G. *Am. J. Med.* **2005**, *118*, 212.
9. Remington, J. S. In *Infectious Disease of the Fetus and Newborn Infant*; Elsevier Saunders: Philadelphia, 2006; Vol. 42. pp 947–1091.

10. Meneceur, P.; Bouldouyre, M.-A.; Aubert, D.; Villena, I.; Menotti, J.; Sauvage, V.; Garin, J.-F.; Derouin, F. *Antimicrob. Agents Chemother.* **2008**, *52*, 1269.
11. Anderson, A. C. *Drug Discovery Today* **2005**, *10*, 121.
12. el Kouni, M. H. *Pharmacol. Ther.* **2003**, *99*, 283.
13. el Kouni, M. H. *Curr. Pharm. Des.* **2007**, *13*, 581.
14. Iltzsch, M. H.; Uber, S. S.; Tankersley, K. O.; el Kouni, M. H. *Biochem. Pharmacol.* **1995**, *49*, 1501.
15. Yadav, V.; Chu, C. K.; Rais, R. H.; Al Safarjalani, O. N.; Guarcello, V.; Naguib, F. N. M.; el Kouni, M. H. *J. Med. Chem.* **2004**, *47*, 1987.
16. Rais, R. H.; Al Safarjalani, O. N.; Yadav, V.; Guarcello, V.; Kirk, M.; Chu, C. K.; Naguib, F. N. M.; el Kouni, M. H. *Biochem. Pharmacol.* **2005**, *69*, 1409.
17. Kim, Y. A.; Sharon, A.; Chu, C. K.; Rais, R. H.; Al Safarjalani, O. N.; Naguib, F. N. M.; el Kouni, M. H. *Biochem. Pharmacol.* **2007**, *73*, 1558.
18. Kim, Y. A.; Sharon, A.; Chu, C. K.; Rais, R. H.; Al Safarjalani, O. N.; Naguib, F. N. M.; el Kouni, M. H. *J. Med. Chem.* **2008**, *51*, 3934.
19. Al Safarjalani, O. N.; Rais, R. H.; Kim, Y. A.; Chu, C. K.; Naguib, F. N. M.; el Kouni, M. H. *Biochem. Pharmacol.* **2008**, *76*, 958.
20. El Kouni, M. H.; Guarcello, V.; Al Safarjalani, O. N.; Naguib, F. N. M. *Antimicrob. Agents Chemother.* **1999**, *43*, 2437.
21. Al Safarjalani, O. N.; Naguib, F. N. M.; el Kouni, M. H. *Antimicrob. Agents Chemother.* **2003**, *47*, 3247.
22. Herdewijn, P.; De Clercq, E.; Balzarini, J.; Vanderhaeghe, H. *J. Med. Chem.* **1985**, *28*, 550.
23. Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319.
24. Wang, P.; Agrofoglio, L.; Newton, M.; Chu, C. K. *J. Org. Chem.* **1999**, *64*, 4173.
25. Wang, P.; Gullen, B.; Newton, M. G.; Cheng, Y. C.; Schinazi, R. F.; Chu, C. K. *J. Med. Chem.* **1999**, *42*, 3390.
26. Jin, Y. H.; Liu, P.; Wang, J.; Das, U.; Baker, R.; Huggins, J.; Chu, C. K. *J. Org. Chem.* **2003**, *68*, 9012.
27. Song, G. Y.; Naguib, F. N. M.; el Kouni, M. H.; Chu, C. K. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1915.
28. Shealy, Y. F.; Clayton, J. D. *J. Am. Chem. Soc.* **1969**, *91*, 3075.
29. Storer, R. *Drug Discovery Today* **1996**, *1*, 248.
30. An, H.; Cook, P. *Chem. Rev.* **2000**, *100*, 3311.
31. Sullivan, W. J., Jr.; Chiang, C. W.; Wilson, C. M.; Naguib, F. N. M.; el Kouni, M. H.; Donald, R. G.; Roos, D. S. *Mol. Biochem. Parasitol.* **1999**, *103*, 1.
32. Schumacher, M. A.; Scott, D. M.; Mathews, I. I.; Ealick, S. E.; Roos, D. S.; Ullman, B.; Brennan, R. G. *J. Mol. Biol.* **2000**, *298*, 875.
33. Zhang, Y.; el Kouni, M. H.; Ealick, S. E. *Acta Crystallogr.* **2007**, *63*, 126.
34. Sherman, W.; Day, T.; Jacobson, M. P.; Friesner, R. A.; Farid, R. *J. Med. Chem.* **2006**, *49*, 534.
35. Maestro, version 8.0; Schrodinger LLC: New York, 2007.
36. Glide, version 4.5; Schrodinger LLC: New York, 2007.
37. Schrodinger Suite 2007; Schrodinger LLC: New York, 2007.
38. MacroModel (Schrodinger Suite 2007), version 9.5; Schrodinger LLC: New York, 2007.
39. Cha, S. *Mol. Pharmacol.* **1968**, *4*, 621.
40. Pfefferkorn, E. R.; Pfefferkorn, L. C. *J. Protozool.* **1977**, *24*, 449.